Session 10 − **Cancer and Cell Proliferation**

10.001

Obesity and cancer development: effect of metformin. Fonseca EAI[1], Oliveira, M. A.[1], Tostes RCA[1], Colquhoun A[2], Carvalho MHC[1], Zyngier SZ[1], Fortes ZB[1] [1]ICB-USP − Farmacologia, [2]ICB-USP − Biologia Tecidual e do Desenvolvimento

Introduction: Epidemiological studies have associated obesity with a wide variety of cancer. Insulin resistance and hyperinsulinaemia may be the mechanisms by which obesity induces or promotes tumorigenesis. Metformin, an antidiabetic drug, can have an antitumoral effect directly or indirectly by improving insulin sensitivity. Therefore, the objective of this study was to analyze the influence of obesity and insulin resistance in the tumor development and, the effect of metformin on it. **Methods:** Obesity was induced in Wistar rats by monosodium glutamate (MSG). Newborn male rats were subcutaneously injected with 400mg/kg MSG (obese) or saline (control) at 2,3,4,5 and 6 days of age. Control (C) and obese-MSG (O) rats of 16 weeks of age received 5x105 Walker-256 tumor cells subcutaneously injected in the right flank (CT and OT). Part of these rats received concomitantly treatment with metformin 300 mg/kg, via gavage (CTM and OTM). The respective controls received tap water (CT and OT). Thus 4 groups were formed: Control tumor (CT), Control tumor metformin (CTM), Obese-MSG tumor (OT) and Obese-MSG tumor metformin (OTM). On the 18th week, obesity was characterized by the Lee index (body weight 1/3 (g)/naso-anal length (cm)), periepididymal and retroperitoneal adipose tissues weight and lipid profile. Insulin sensitivity was evaluated by plasma glucose disposal rate (Kitt; %/min) and the lipid peroxidation rate by TBARS assay. The percentage of tumor incidence, tumor relative weight and the percentage of cachexia incidence were also analyzed. The tumor tissue was evaluated histologically by means of hematoxylin and eosin staining. **Results:** The tumor incidence (OT 82*** vs CT 55%), and the tumor relative weight (OT 8.9±0.9*** vs CT 5,5±0,5 g/100g body weight) were significantly higher in the OT. Both parameters were reduced by metformin treatment (CTM 56, OTM 59##% and, CTM 4,1±0,5, OTM 5,1±0,6##g/100g weight , n=16, respectively). The cachexia incidence was higher in the OT group than in the other groups and metformin did not correct this parameter (OT 90*** vs CT 50, CTM 50, OTM 100%, n=18). The tumor tissues were qualitatively similar in all groups. As expected adipocytes were only found in OT and OTM groups. The necrotic area appeared to be greater in the groups treated with metformin than in the other groups. Metformin did not correct the insulin resistance in OT (Kitt OT 1,95±0,18*, OTM 2,43±0,18*, CT 3,07±0,28, CTM 3,45±0,27%/min, n=8), however it did correct the dyslipidemia, reduced the periepididymal and retroperitoneal adipose tissues (OT 2,4±0,11*, OTM 2,15±0,08#, CT 1,32±0,05, CTM 1,00±0,05* g/100g weight, n=10 and OT 2,67±0,11*, OTM 2,14±0,13#, CT 1,17±0,08, CTM 0,58±0,065* g/100g weight, n=10, respectively) and reduced the lipid peroxidation rate. **Discussion:** Metformin was able to reduce the incidence and Walker-256 tumor development but not cachexia in MSG obese rats. The reduction occurred independently of the correction of insulin resistance, since insulin sensitivity was not improved by metformin treatment. ***p<0,001 vs CT, *p<0,05 vs CT, #p<0,05 vs OT e ##p<0,0001 vs OT. Nº da Licença do Comitê de Ética: 007/04/CEEA **Financial support:** CNPq (Brazil), FAPESP (Brazil- Project No 2008/50933-5/ 2007/58311-0), INCT Obesity and Diabetes/CNPq.

10.002

Effects of $P2X_7$ receptor agonists on cell proliferation of human glioma cell lines U138-MG and M059J. Gehring MP[1], Campos MM[2], Battastini AMO[3], Morrone FB[4] [1]PUCRS − Farmacologia Aplicada, [2]PUCRS − Cirurgia-Odontologia, [3]UFRGS − Bioquímica, [4]PUCRS − Farmácia

Introduction: ATP is an important signaling molecule in the peripheral and central nervous system (CNS). Studies have shown that extracellular nucleotides/nucleosides induce proliferation of different glioma cell lines through mechanisms involving P1 and P2 receptors, and treatment with ATP promotes cell cycle progression in human glioma cells. Furthermore, P2X7 expression was found up-regulated under several pathological conditions, including cancer. Recent studies have shown that C6 and U138-MG glioma cells resist to death induced by high concentrations of ATP (5 mM) and BzATP (P2X7 receptor agonist). The present study aimed to compare the effects of P2X7 agonists in the proliferation of human glioma radiosensitive M059J and radioresistant U138-MG cell lines. **Methods:** Glioma cells were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% (U138-MG) and 10% (M059J) fetal bovine serum (FBS), under ideal conditions of cultivation. Cells were plated in 24 and 96 wells in densities of 20 x 103 and 5 x 103 cells/well, respectively. After reaching semi-confluence, cells were treated with ATP (0.5, 3 and 5 mM) and BzATP (5 to 100 μM). To assess cell proliferation, cells were counted in hemocytometer, and cell viability was assessed by the MTT assay, at 24, 48 and 72 h. All experiments were carried out at least three times in triplicate. Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey-Kramer test. **Results:** The human glioma line U138-MG presented resistance to death when treated with either ATP or BzATP, whereas the high ATP concentration (5mM) and the selective P2X7 agonist BzATP, in a concentration as low as 5 μM, significantly diminished the cell viability (61.7% and 17.8%, respectively) of the glioma line M059J. **Discussion:** Our results showed, for the first time, that treatment with high ATP concentration and BzATP induced cell death of the human glioma cell line M059J. In agreement to previous studies, the human cell line U138-MG showed resistance to death when treated with the same agonists. Our data indicate the possible participation of the receptor P2X7 on the cell death of radiosensitive glioma cells. **Financial support:** CAPES and PUCRS.

10.003

Evaluation of butyrate and aqueous extract of the *Ilex paraguariensis* enemas in reducing the levels of malondialdehyde in exclusion of colitis. Silva CMG, Lameiro TMM, Marques LHS, Almeida MG, Cunha FL, Martinez CAR − Sao Francisco University − Cancer and cell proliferation

Introduction: The deficiency of butyrate in the excluded colon determining oxidative stress is related to the pathogenesis of diversion colitis. However, no studies have evaluated the efficacy of butyrate and aqueous extract of the Ilex paraguariensis in reducing tissue levels of oxidative stress in experimental models of diversion colitis. The aim of this study was evaluated the therapeutic effects of butyrate and aqueous extract of Ilex paraguariensis enemas in reducing levels of oxidative stress in diversion colitis. **Methods:** The study was approved by Ethics Committee on Animal Research of São Francisco University (nº 002.04.10). Thirty-two rats were divided into two experimental groups with 16 animals. They were sacrificed two or four weeks after completion of terminal colostomy in the descending colon and distal mucous fistula. Each experimental group was divided into two subgroups according to application (sub-experiment) or not (control subgroup) from enemas in the excluded colon, with butyrate at a concentration of 100mg/kg or 150mg/kg of the aqueous extract of Ilex paraguariensis. The diagnosis of colitis was made by histopathology analysis and the levels of oxidative stress by measuring the tissue levels of the malondialdehyde by spectrophotometry. The results were analyzed with Mann -Whitney, taking significance level of 5% ($p < 0.05$). **Results and Discussions**: The tissue levels of malondialdehyde in the colon with and without fecal stream, after two and four weeks, animals underwent rectal irrigation with butyrate and aqueous extract of the Ilex paraguariensis were: The levels of malondialdehyde in the control animals after two and four weeks of irrigation were 0.05 ± 0.006, 0.06 ± 0.006 and 0.05 ± 0.03, 0.08 ± 0.02 respectively. Malondialdehyde levels in the animals irrigated with butyrate in the colon with and without fecal stream, after two and four weeks of irrigation were 0.05 ± 0.01 - 0.04 ± 0.01 and 0.04 ± 0.01 - 0.04 ± 0.01, respectively. After two and four weeks of intervention the malondialdehyde levels were lower in the animals irrigated with butyrate, regardless of time of irrigation ($p = 0.003$ $p = 0.001$, respectively). Malondialdehyde levels in the animals irrigated with Ilex paraguariensis, in the colon with and without fecal stream, after two and four weeks of irrigation were 0.010 ± 0.002, 0.02 ± 0.004 and 0.03 ± 0.007, 0.04 ± 0, 01, respectively. After two and four weeks of intervention levels of malondialdehyde were lower in the animals irrigated with Ilex paraguariensis, regardless of time of irrigation ($p = 0.0001$ and $p = 0.002$, respectively). These results suggest that rectal interventions with antioxidant substances like butyrate and Ilex paraguariensis show beneficial therapeutic effects in treatment of diversion colitis. The results of the present study confirm that the use of rectal enemas of substances with recognized antioxidant activity can reduces levels of oxidative stress tissue damage confirming the importance of oxygen free radicals in the pathogenesis of diversion colitis. Acknowledgements: CAPES, FAPESP (Process number: 2006-02306-6) and São Francisco University for financial support.

10.004

Glucose starvation induces melanogenesis in B16F10 murine melanoma cells through oxidative stress. Piva B[1], Diaz BL[2] IBCCF-UFRJ − Programa de Imunobiologia

Introduction: Tumor cells have defects in their regulatory circuitry that govern cell proliferation and homeostasis [1]. In a tumor mass, vasculature is disorganized leading to poor distribution of oxygen and nutrients that induces high stress levels in cells. Such stress is able to make cells more resistant to chemotherapy [2, 3]. Melanoma resistance to chemotherapy may be related to its origin in transformed melanocytes and its inherent ability to synthesize melanin in response to stress such as UV radiation that may provide protective capacity for the melanoma cell. Melanin is a molecule that possesses several properties [4] and may provide protective functions for the melanoma cell. The objective of this study was to analyze the response of B16F10 cells to glucose deprivation, including melanogenesis, cell proliferation and viability, and modulation of signaling pathways. **Methods:** B16F10 murine melanoma cells were maintained in complete DMEM high glucose (4,5 g/L). Nutrient stress was induced by replacing the medium with no glucose DMEM. After different periods of time cells were harvested and viability was assessed by trypan blue exclusion and annexin V staining while melanogenesis was evaluated by determining Tyrosinase activity and melanin content spectrophotometrically. Morphological analysis were made with phalloidin and visualized by fluorescence microscopy. Nutrient stressed cell were treated with pyruvate, antioxidants, such as N-acetylcysteine or reduced glutathione, or SB202190 (p38 inhibitor). **Results:** B16F10 cells cultured in DMEM without glucose for 24 hours, showed 2-fold induction of tyrosinase activity followed by melanogenesis. Cells cultured without glucose for 24 hours also showed 95% reduction in proliferation rate and reorganization of actin filaments, but with no effect on cell viability. Supplementation of culture medium with pyruvate prevented (100% inhibition) melanogenesis in glucose-deprived cells. Nutrient stress-induced melanogenesis was also inhibited when anti-oxidant capacity of cells was increased by N-acetylcysteine or reduced glutathione treatment, 100% and 50% of inhibition respectively. Cells treated with SB202190, a p38 inhibitor, exhibited an increase of 50% in melanin synthesis when cells were cultured under glucose deprivation. **Discussion:** In conclusion, glucose deprivation is a potent stress stimulus for B16F10 leading to melanogenesis, proliferation arrest and morphological changes due to oxidative stress. Concomitant p38 activation seems to counteract the effects of nutrient stress in B16F10 cells. References: 1- Hanahan D. Cell, 100: 57 (2000). 2- Rofstad E.K. Br. J. Cancer, 80: 1697 (1999). 3- Trédan O. J. Natl. Cancer Inst. 99: 1441(2007). 4- Simon J.D. J. of Phys. Chem., 112: 13201 (2008). This work was supported by CNPq and FAPERJ.

10.005

Structure-related activity of a series of chalcones derived from quinoxaline on *in vitro* oral squamous cell carcinoma proliferation. Mielcke TR[1], Mascarello A[2], Calixto JB[3], Yunes RA[2], Leal PC[2], Morrone FB[4], Campos MM[5] [1]PUCRS − Farmacologia, [2]UFSC − Química, [3]UFSC − Farmacologia, [4]PUCRS − Farmácia, [5]PUCRS − Cirurgia-Odontologia

Introduction: Oral squamous cell carcinoma (OSCC) is a neoplasia that accounts for more than 90% of all oral cancers (INCA, 2002; Bagan J, Oral Oncol, 2010 fev). Cancers of the oral cavity represent a health problem, as indicated by their high incidence in Brazil and in many parts of the world (INCA, 2002). Despite the significant advances in treating other types of malignancies, the morbidity and mortality associated with oral cancer remains high (Brinkman and Wong, Curr Opin Oncol 2006, 18:228; McDowell, Otolaryngol Clin N Am 2006, 29:277). Chalcones are a group of natural compounds identified in many different plant organs (Ducki, IDrugs, 2007, 10(1):42; Kontogiorgis, Mini Rev Med Chem,2008, 8(12):1224). They are precursor compounds for flavonoid synthesis and display a wide variety of biological and pharmacological proprieties that include anti-proliferative and anti-cancer activities (Boumendiel A, J Med Chem 2008, 51:2307). In the present study, we have examined the *in vitro* effects of a series of nine synthetic quinoxaline-derived chalcones on the viability of OSCC cell lineages. **Methods:** After the rat oral squamous carcinoma cell line SCC 158 reached confluence, they were seeded at 1 x 103 cells/well in DMEM/10 % FBS, in 96-well plates. Then the cells were exposed to increasing concentrations (0.1 - 10 µg/ml) of the test compounds, for incubation periods of 24 h, 48 h and 72 h. The tested chalcones are denoted as: N2, N3, N4, N5, N6, N7, N9, N10 and N12. Parallel control experiments were carried out with the addition of 10 % FBS (cell viability control) or DMSO (vehicle control, 0.01 %), in the absence of chalcones. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2yl)- 2,5-diphenyl tertrazolium bromide (MTT) assay. It provides a quantitative measure of the number cells with metabolically active mitochondria. Results and **Discussion:** All the evaluated chalcones produced a significant decrease of SCC158 rat squamous carcinoma cell line viability, but four of them (N2, N9, N10 and N12) displayed a higher inhibitory effect. The maximum inhibition (Imax) for these chlacones were 56 ± 7 %, 60 ± 5 %, 58 ± 5 % and 55 ± 12 %, respectively. The estimated IC50 values (µg/ml, accompanied by the confidence interval) were 4,42 (4,03 – 4,84); 2,66 (2,09 – 3,38); 4,16 (3,51 – 4,93) and 4,60 (4,07 – 5,20), correspondingly. The inhibitory effect revealed to be concentration-dependent. In addition, the effects of these four chalcones were found to be time-dependent, being maximal between 48 and 72 h of *in vitro* incubation. **Conclusion:** These results clearly suggest that chalcones derived from quinoxaline might represent promising molecules for the treatment of oral squamous carcinoma progression, although the in vivo efficacy of these compounds remains to be confirmed in future studies. Furthermore, it is obviously necessary to clarify the mechanisms implicated in the anti-tumor effects of the quinoxaline-derived compounds tested in our study. **Financial support:** CNPq, PUCRS.

10.006

Stress-related neurohormonal mediators influence the human oral cancer cells behavior. Bernabé DG[1], Tamae AC[1], Miyahara GI[2], Biasoli ER[2], Oliveira SHP[1] [1]FOA-UNESP − Ciências Básicas, [2]FOA-UNESP − Ciências Básicas, [3]FOA-UNESP − Oncologia Bucal

Introduction: Little is known about the interference of stress-related neurohormones factors in the oral squamous cell carcinoma (OSCC). In this study, we have investigated the effects of stress hormones on OSCC-derived cell lines. **Methods:** The effects of the norepinephrine (NE), isoproterenol, and cortisol on Interleukin-6 (IL-6) expression were studied in SCC9 and SCC25 cells. IL-6 expression was evaluated by real time PCR, and protein production was assessed by ELISA. The effect of hormones on cell proliferation was examined by MTT. Expression of ß-adrenergic receptors (ß-ARs) was studied by real time PCR in the cell lines, as well as in 20 samples of OSCC, 17 samples of leukoplakia, and 15 samples of normal oral mucosa. Results. Real time PCR studies revealed constitutive ß1 and ß2-adrenergic receptors (ß-ARs) expression in the SCC9 and SCC25 cells. The results showed that NE and isoproterenol significantly enhanced IL-6 mRNA expression and protein production in supernatants of SCC9 and SCC25 cells. Physiological stress levels of NE and isoproterenol (10 µM) at 1 hour elicited the most robust IL-6 increase. In these conditions, NE induced an increase of 501.5% ± 34.8% and 237.7% ± 37.6% in IL-6 mRNA expression in SCC9 ($p<0.001$) and SCC25 cells ($p<0.05$), respectively. Regarding IL-6 secretion, 10 µM NE induced a 5-fold increase at 1 hour, 3.7-fold increase at 6 hours and 3.2-fold at 24 hours in SCC9 cells. These effects were blocked by the ß-adrenergic antagonist propranolol, supporting a role for ß-ARs in IL-6 secretion. The effects of cortisol varied according to the cell line and hormone concentration. Pharmacological concentrations of cortisol (1000 nM) inhibited IL-6 production by SCC9 and SCC25 cells. Cortisol dose that simulates stress conditions (10 nM) tended to increase IL-6 expression in SCC9 cells. NE (10 µM, at 6 hours) and cortisol (1000 nM, at 48 hours) stimulated increase proliferation of SCC9 cells. All OSCC samples expressed ß1 and ß2-ARs. Quantitatively, ß1-AR was more expressed in OSCC samples, whilst ß2-AR expression was lower in leukoplakia. **Discussion:**. These findings provide direct experimental evidence that stress-related neurohormonal mediators can affect OSCC progression.
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10.007

Anticancer Activities of 2,2-Dimethyl-3-(3-nitrophenylamino)-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (1): Oxidative Stress based-apoptosis. Araújo AJ[1], Marinho-Filho JDB[1], Silva-Junior EN[2], Moura MABF[3], Goulart MOF[4], Ferreira VF[2], Pessoa C[1], Moraes MO[1], Costa-Lotufo LV[1], Montenegro RC[1] [1]UFC − Fisiologia e Farmacologia, [2]UFF − Química Orgânica, [3]UFAL − Química, [4]UFAL − Química e Biotecnologia

Introduction: The development of new chemotherapeutic for the treatment of cancer is urgently needed, and knowledge of their pharmacological mechanism of action is crucial for their clinical use. Drugs containing quinone moiety are known to show anticancer activity. Therefore, the aim of this work was to evaluate the anticancer activity of the compound 2,2-dimethyl-3-(3-nitrophenylamine)-2,3-dihydronaphtho[1,2-b]furan-4,5-dione (QPhNO2) and its precursor, nor-beta-lapachone (nor-beta). **Methodology**: The compounds were tested against HL-60 cells (leukemia) using MTT assay, after 24 hours of incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. The inhibition of proliferation was also determined by DNA synthesis, based on the reduction of BrdU incorporation, using HL-60 as model. To further understand the mechanism underlying the cytotoxicity of QPhNO2 and its precursor, the differential morphology tests with May-Grünwald Giemsa and acridine orange/ethidium bromide staining were analysed and studies involving DNA fragmentation, cell cycle analysis, phosphatidyl serine (PS) externalization, mitochondrial depolarization, caspases activation and measurement of ROS were performed by flow cytometry in HL-60 cell line, using doxorubicin as a positive control. Results and **Discussion:** The IC50 values of QPhNO2 and nor-beta were 0.48 and 2.92 μM, respectively. To investigate the possible mechanisms involved in the cytotoxicity of QPhNO2 and its precursor, nor-beta, HL-60 cells were used as a model based on the values of its IC50 after 24 h of incubation. QPhNO2 was tested at concentrations of 0.5, 1.0, and 2.0 μM and nor-beta at 1.0 and/or 2.0 μM. QPhNO2 caused DNA fragmentation, mitochondrial depolarization and differential morphology staining indicating the induction of apoptosis. In cells treated with nor-beta, at all tested concentrations, no differences were observed when compared with untreated cells. PS externalization revealed that QPhNO2 at 2.0 μM induced apoptosis on 32.87% of the cells, whereas nor-beta induced only 12.49%. Necrosis was observed in 7.90% of the cells treated with QPhNO2. Caspases activation was observed in cells treated with QPhNO2, whereas no caspase activation was observed in cells treated with nor-beta. With regard to the cell cycle, no difference was observed in either of the tested compounds. Both tested compounds stimulated ROS generation after 1 and 3 h of incubation. However, ROS generation was higher in the first hour than after 3 h of incubation. The apoptosis observed may be caused by the release of ROS after quinone reduction that leads to a dianion-biradical [Q●-]-PhNO2●-. This electrogenerated species can react with oxygen, generating ROS and promoting an oxidized environment that could compromise mitochondria, provoking apoptosis. These findings point to the potential use of the promising field of pharmacoelectrochemistry in Medicinal Chemistry. Supported by: CNPq, IM/INOFAR, CAPES, CNPq/Neoplasias, FUNCAP, FINEP and InCb.

10.008

Effect of crotoxin, the main toxin of the rattlesnake C.*d*. *terrificus* venom, on secretory activity of peritoneal macrophages during tumor progression. Studies *in vivo* and *in vitro*. Costa ES[1], Faiad OJ[1], Curi R[2], Cury Y[1], Sampaio SC[1] [1]IBu − Fisiopatologia, [2]ICB-USP − Fisiologia e Biofísica

Introduction: Crotoxin (CTX) inhibits tumor growth and modulates the function of macrophages. Despite this evidence, the contribution of macrophage inhibition to the decrease in tumor growth, caused by CTX, was not determined yet. Macrophages provide a defense mechanism against tumor cells and two distinct polarization states, M1 and M2, have been described for these cells. In the beginning of tumor progression, M1 macrophages release reactive nitrogen/oxygen intermediates and the cytokines TNF-α, IL-1β and IL-6. In contrast, during tumor development, the release of these mediators by tumor-associated macrophages (M2 cells) is inhibited, contributing to tumor development. In the present study was evaluated the effect of CTX on the activity (H2O2, nitric oxide-NO and cytokines release) of macrophages obtained from peritoneal cavity of Walker 256 tumor-bearing rats (in vivo assay, Ethical Committee For animal Research of Butantan Institute no 495/08) or macrophage co-cultivated with LLC WRC 256 tumor cells (*in vitro* assay, Ethical Committee For animal Research of Butantan Institute no 631/09). **Methods:** In *in vivo* study, male Wistar rats were inoculated (2x107, s.c., in the rigth flank) with tumor cells and treated with CTX (18µg/300µl/rat, s.c.) on day 1 (M1 macrophage) or 5 (M2 macrophage) after cell injection. Macrophages were obtained on day 14 after cell inoculation. *In vitro* assay, the effect of CTX on the production nitric oxide-NO of macrophages co-cultivated with LLC WRC 256 tumor cells was investigated. Macrophages were obtained from peritoneal cavity and cells (2x105) were incubated with CTX (0,3µg/mL) for 2 h at 37oC. After this time, the macrophages were co-cultivated in presence of LLC WRC 256 tumor cells (2x104), previously plated in 96-well culture dishes. After 48 h at 37oC, in a humidified atmosphere of 5% CO2 in air, the effect of CTX on the production nitric oxide-NO was evaluated. **Results:** In vivo, CTX (day 1 and 5, respectively) stimulates H2O2 (149% and 162%) and NO (77% and 94%) production and the release of the cytokines IL-1 β (day 1: 71%) and TNF- α (day 5: 1.59 fold). *In vitro*, the results showed that macrophages previously incubated in the presence of CTX and co-cultivated with tumor cells generated a greater quantity of NO (75%) than control macrophage. **Discussion:** The results indicate that CTX modifies the secretory activity of M2 cells, in vivo and *in vitro* assay, which may contribute to the inhibitory action of the toxin on tumor growth. These data reinforce the actions of CTX on defense mechanisms and bring new perspectives for the development of a new substance with therapeutic properties. Supported by FAPESP (08/53840-8; 09/52330-9), CNPq/PIBIC and INCTTX.

10.009

Cytotoxic activity of benzothiazole analogues. Vieira GC[1], Araújo AJ[1], Vasconcelos, TRA[2], Ferreira VF[2], Nogueira, AF[2], Pessoa CO[1], Costa-Lotufo LV[1], Montenegro RC[1], Moraes MO[1] [1]UFC − Fisiologia e Farmacologia, [2]UFF − Química Orgânica

Introduction: Benzothiazoles are compounds with one benzene ring linked to a thiazole ring. In current scientific literature it has been found that some derivatives of Benzothiazoles had demonstrated to be useful in the treatment of innumerable illnesses, mainly cancer. Samples of Benzothiazoles analogues were studied in selected tumor cell lines observing the type of predominant cellular death. Methodology: The analogues were tested against four cancer cell lines: HL-60 (leukemia), MDA/MB-435 (melanoma), HCT-8 (colon) and SF-295 (brain) using Alamar Blue assay, after 72 hours of incubation. To perform the hemolytic assay, a 2% mouse erythrocyte suspension was used. After incubation for 1h with compounds (0.78-200 µg/mL), the supernatant containing hemoglobin was measured at 540 nm. To further understand the mechanism underlying the cytotoxicity of Benzothiazole, differential morphology tests with May-Grünwald Giemsa and acridine orange/ethidium bromide staining were analyzed in HL-60 cell. Results and **Discussion:** The sample that presented greater cytotoxic activity amongst all the analyzed samples was 2- {(E) - [2-(1,3-benzotiazol-2-il) hidrazinilideno] metil} - 4-nitrofenol, where its value for IC50 ranged from 1,65 to 14,01µM between the tumor cell lines. It was found that this analogue has the capacity to reduce cell viability of HL60 cells superior to that observed for doxorubicin. The observed cellular death presented characteristics of apoptosis although it also presented some necrotic activity. The experiments carried through in this study cannot yet affirm the type of cellular death that the studied analogue induces and nor the mechanism that is involved. More analyses need to be carried through to better know the activity of this analogue. Supported by: CNPq, IM/INOFAR, CAPES, CNPq/Neoplasias, FUNCAP, FINEP and InCb.

10.010

Assessment of the cytotoxic, antiproliferative, genotoxic, mutagenic and pro-apoptotic activities of indirubin, directed for its use as an anticancer substance. Fogaça MV[1], Cardoso PF[1], Cândido PM[1], Varanda EA[2], Calvo TR[3], Vilegas W[3], Cólus, IMS[1] [1]UEL− Biologia Geral, [2]FCFAR-UNESP − Ciências Biológicas, [3]IQ-UNESP − Química

Introduction: Higher plants belonging to the species Indigofera suffruticosa and I. truxillensis present compounds with therapeutic properties, such as the bis-indole alkaloid indirubin, which is used to treat myelogenous leukemia in Chinese medicine due to its antiproliferative activity in tumour cells. However, more studies are needed to verify the anticancer potential of this substance and the security of its use. Thus, the objective of this study was to evaluate the cytotoxic, antiproliferative, mutagenic, genotoxic and pro-apoptotic potentials of indirubin in mammalian cells. **Methods:** Chinese hamster ovary cells (CHO-K1) and epithelial cells of human cervical carcinoma (HeLa) were treated with different concentrations of indirubin to perform the MTT test (0.1, 0.5, 1.0, 5.0, 10.0, 25.0, 50.0, 100.0 and 200.0 µM) (Mosmann TJ, Immunol Methods, v.65, p.55, 1983), the cytokinesis-block micronucleus assay (MN) (Heddle JA, p.191, 1976; Fenech M, Citobios, v.43, p.233, 1985) and the apoptosis test (0.1 to 10.0 µM) (McGahon A.J, Methods Cell Biol, v.46, p.153, 1995). Five groups of Swiss albino mice (Mus musculus) (8/group, 4 males and 4 females) were used to perform the comet (Singh NP, Exp Cell Res, v.175, p.184, 1988) and the micronucleus (Hayashi M, Mut Res, v.245, p.245, 1990) assays with acute exposure to the drug (50.0, 100.0 and 150.0 mg/kg, administered via gavage). All animal procedures were approved by the Ethics Committee of the State University of Londrina (CEEA/UEL 08/09, protocol nº 42492/08). As positive control it was used doxorubicin (0.75 µg/mL of medium culture) and cyclophosphamide (40.0 mg/kg) and as negative control it was used phosphate buffered saline. Statistical analyses were performed by one-way ANOVA followed by Tukey test. Results and **Discussion:** Indirubin was more cytotoxic to tumor cells (5.0 to 200.0 µM, $p<0.001$) than to CHO-K1 cells (10.0 to 200.0 µM, $p<0.001$) in the MTT assay. The MN and apoptosis *in vitro* tests did not indicate, respectively, mutagenic and pro-apoptotic activities of indirubin at all the concentrations tested ($p>0.05$). The nuclear division index achieved by the MN test showed antiproliferative activity of the substance for both cell lines (CHO-K1: 0.5 to 10.0 µM, $p<0.05$; HeLa: 5.0 to 10.0 µM, $p<0.05$). It was observed genotoxic effect of indirubin in the comet assay (100.0 and 150.0 mg/kg, $p<0.05$). However, the compound was not mutagenic to polychromatic reticulocytes ($p>0.05$), suggesting that the lesions induced in DNA and detected by the comet assay may have been effectively repaired. **Conclusion:** Therefore, indirubin can contribute in the future as an anticancer substance. Although the compound had been more selective for tumour cells in the cytotoxicity test and had shown antiproliferative properties and lack of mutagenicity in mammalian cells, still needs further studies before being recommended for human use, since it was observed genotoxicity in the comet assay. **Financial support:** Biota-Fapesp, CNPq-PQ, Fundação Araucária

10.011

Characterization of cytotoxic activity induced by phospholipase A2 (PLA-LYS49) isolated from *Bothrops jararacussu* snake venom. Lino CNR[1], Sorgi CA[2], Cintra ACO[2], Sampaio SV[2], Faccioli LH[2], Nomizo A[2] [1]FFCLRP-USP − Biologia, [2]FCFRP-USP − Análises Clínicas, Toxicológicas e Bromatológicas

Introduction: Phospholipase A2 (PLA2) is a super family of enzymes that hydrolyzes fatty acids from the sn-2 position of glycerophospholipids from cellular membranes to generate free fatty acids [1]. Some PLA2 which has a lysine in the 49 position (Lys 49), instead of the aspartate, show low or no catalytic activity, but maintain its cytotoxic activity such as Bothropstoxin-I (BthTX-I) from Bothrops jararacussu [2]. This work intended to evaluate the antitumor effect of the BthTX-I in different cells lineages, also evaluating the type of death induced by it (necrosis or apoptosis). **Methods**: The cells lines JURKAT, K562, HL60, HL60-Bcl2 and B16-F10, were used in present study. The cytotoxic activity of the BthTX-I was assessed using MTT assay. To analyze the cell morphology, K562 cells were incubated with BthTX-I and stained with panoptic or DAPI. The cell death type (apoptosis or necrosis) was assessed staining the cells with annexin V and propidium iodide (PI) and analyzed by flow cytometry. **Results and Discussion:** The cytotoxic effect of the BthTX-I was observed in all the cell lineages in a dose dependent manner. The percentage of death showed in JURKAT, K562, HL60, HL60-Bcl2 and B16-F10 cells at the top concentration used in the experiments (160 µg/ml) were respectively: 62.30%, 70.70%, 33.26%, 25.86% and 79.22%. The K562 cells treated with BthTX-I exhibit cell membrane disruption, suggesting necrosis, in contrast, treatment with Camptotecin induces reduction of the relation nucleus/cytoplasm and reduction of pigmentation of the nucleus, suggesting apoptosis. Furthermore, to confirm the type of cell death induced by BthTX-I, was assayed a FACS analysis. K562 cells treatment with BthTX-I for 3 h already showed an expressive number of necrotic cells (45.7% at 40 µg/ml and 69.7% at 80 µg/ml). After 9 h of treatment the percentage of necrosis was practically maintained at 40 µg/ml (47%), and increased at 80 µg/ml (79.8%). The percentage of apoptotic cells was kept low through the time. These results indicated that BthTX-I exhibit cytotoxic activity in tumor cells as dose dependent manner and also induces cell death almost always by inducing necrosis. Finally, the results showing that BthTX-I exhibit cytotoxicity on HL60-Bcl2 cell line, suggested that the class of PLA2 as BthTX-I must be important to kill tumor cells lines that presented refractivity to apoptosis by over expression of Bcl2 protein [3]. **References**: 1-Schaloske RH et al., Biochim Biophys Acta. 1761: 1246–1259, 2006. 2-Ward RJ et al., Protein Expr. Purif. 21: 134-140, 2001. 3-Yang J et al., Science, 275: 1129 - 1132, 1997. **Funding:** PIBIC-RUSP.

10.012

Involvement of the kinin B1 receptor in melanoma progression. Maria AG[1], Reis RI[1], Dillenburg-Pilla P[1], Pesquero JB[2], Costa-Neto CM[1] [1]FMRP-USP − Bioquímica e Imunologia, [2]UNIFESP − Biofísica

Introduction: Melanoma is one of the most deadly human cancers with no effective cure for metastatic disease and its incidence is increasing rapidly. Thus, identification of genetics determinants that drive melanoma formation and progression is crucial to find new insights for drug therapy. The kinins, bradykinin and Lys-des[Arg9] bradykinin are important inflammatory mediators that act by binding to two receptor types, B2 and B1 (B2R, B1R) respectively. These receptors belong to the G-protein coupled receptor family. B1 receptor generally is not expressed at significant levels in healthy tissues, but its expression is increased under pathological conditions. In this study, we investigated the involvement of B1R in melanoma progression and possible signaling pathways that it might be involved. **Methods:** Wild type (WT) and kinin B1 receptor knockout (B1KO) mice were inoculated with B16/F10 murine melanoma cells and tumors were collected 22 days after cells inoculation. PCR assays for tumor samples were performed for B1R, B2R and angiotensin converting enzyme (ACE). We also performed wound healing assay for B16/F10 cells. The activation of p53, Akt, p38 and ERK1/2 pathways were analyzed by western blot. All experiments were conducted in accordance with the local Animal Care and Use Committee (FMRP-USP 121/2009). **Results:** Concerning to tumor growth and volume, there was no significant difference between B1KO and the WT group. However, around 50% of B1KO animals presented skin disruption near to tumor area, while only 10% of the WT animals presented this feature. Wound healing assay showed that cells treated with B1R agonist, decrease their capacity of migration, which could represent a protective mechanism of the cell against tumor progression. Concerning to mRNA analysis, the levels of B1R expression in B1KO tumor samples was decreased in 50% comparing to the WT tumor samples. We also found an increase of 2 folds in ACE mRNA levels of expression in B1KO tumor samples compared to WT samples. Phosphorylation of p53 increased 1.8 folds in B1KO tumor samples and also, these samples showed 2.2 folds of increase of ERK1/2 phosphorylation and 1.6 folds of increase of Akt phosphorylation compared to the WT tumor group. However, levels of p38 phosphorylation in BIKO tumor samples decrease 60% comparing to WT tumor samples. **Discussion:** Our results comparing B1KO and WT tumor samples suggest that B1R and its interaction with the tumor microenvironment could be involved in several signaling pathways activation. The decrease of B1R mRNA expression in tumor samples could trigger cell apoptosis through p53 activation, indicating DNA damage and genomic instability of the cells. In addition, B1KO mice developed tumors that could increase the activation of different cell proliferation pathways as PI3K/Akt and MAPK/ERK, which lead us to suggest that the lack of B1R contributes to increase the tumor malignancy. However, further studies with tumors in higher stages are needed to provide more information about the participation of B1R in melanoma progression. **Financial support:** FAPESP, CAPES, CNPq, FAEPA.

10.013

Overexpression of platelet-derived growth factor receptor-α in basal-like triple-negative breast cancer. Melo-Filho AF[1], Ribeiro RA[2], Rodrigues EJM[2], Magalhães HO[1], Soares FA[3], Chagas DWN[2], Lima VCC[4] – [1]ICC − Mastologia, [2]UFC − Fisiologia e Farmacologia, [3]HCANC − Anatomia Patológica, [4]HCANC − Oncologia Clínica

Introduction: Breast cancer (BC) is a heterogeneous disease with different morphologies, molecular profiles, clinical behavior and response to therapy. The triple-negative breast cancer (TNBC) is a particular type of BC defined by absence of estrogen and progesterone receptor (ER and PR) expression as well as absence of ERBB2 amplification. It is characterized by its biological aggressiveness, worse prognosis and lack of a therapeutic target in contrast with positive hormonal receptor and ERBB2+ BCs. A new molecular classification to BC emerged recently in the scientific scene based in gene expression profiles. The new subgroups (luminal, ERBB2, normal-like and basal-like) have distinct gene expression patterns and phenotypical characteristics. TNBC shares phenotypical features with basal-like BC (BLBC), which is in turn the most aggressive and with worse outcome. Since microarray gene-expression assays are only used in the research setting, clinicians tend to use the TN definition as a surrogate of BLBC. However it has been shown that not all TNBC are BLBC; these tumors are defined as non-basal like (NBL). We lack a specific, easy-to-use method, such as immunohistochemistry patterns, to define BLBC. Platelet-derived growth factor (PDGF) is one of the numerous growth factors that regulate cell growth and its division; it is present in normal and cancerous cells. Platelet-derived growth factor receptor (PDGFR) is a receptor with intrinsic tyrosine kinase activity and deregulated in several human diseases. It has two subunits (PDGFR-α and PDGFR-β). BCs are known to express PDGF; however, there have been few studies on PDGF receptors in breast neoplasias, most of them related to the β subunit (PDGFR-β). In addiction there are no reports with these markers using TNBC subpopulation. Then, we aimed to investigate the potential significance of PDGF and the expression of its two receptor subunits (PDGFR-α and PDGFR-β) in TNBC in order to differentiate BLBC and NLBC. **Methods:** In a tissue microarray (TMA), obtained from a series of 74 formalin-fixed paraffin-embedded samples of TNBC, we used immunohistochemistry to detect PDGF and its receptor subunits (PDGFR-α, PDGFR-β) overexpression. The specimens were obtained from the histopathology files of Cancer Hospital of the Cancer Institute of Ceará. The protocols were approved by the Ethics Committee of this institution (protocol: 036/2009). **Results:** PDGFR-α expression was observed in 79.7% of the TNBC (59 of 74 samples), PDGFR- β in 48.6% of the TNBC (36 of 74 samples) and PDGF in 64.9% of the TNBC (48 of 74 samples). In addition, PDGFR-α was significantly expressed ($P = 0.004$) in 92.1% (35 of 38 samples) of BLBC vs NBL. Moreover, PDGF was expressed in 71.1% of the BLBC (27 of 38 samples) which differs in a significant manner ($P = 0.014$) from NBL. On the other hand, PDGFR- β was expressed in only 51.9% of the BLBC (14 of 27 samples; $P>0.05$ vs NBL). **Discussion:** These data show for the first time that the immunohistochemical detection of PDGF and PDGFR-α expression would be a potential tool to distinguish basal-like to non basal-like TNBC. These findings merits further molecular investigation in order to sustain PDGFR- α as promising therapeutic target in TNBC. **Financial support:** Pronex - CNPq/Funcap

10.014

Leptin activates the mTOR pathway in epithelial cells: roles in lipid metabolism, inflammatory mediator production and cell proliferation. Fazolini NPB FIOCRUZ − Fisiologia e Farmacodinâmica. Fazolini NPB[1], Viola JPB[2], Maya-Monteiro CM[1], Bozza PT[1]. [1]IOC-FIOCRUZ − Imunofarmacologia, [2]INCa – Cellular Biology

Introduction and Aims: Accumulating evidences suggests that obesity and enhanced inflammatory reactions are predisposing conditions for developing colon cancer. However, the molecular mechanisms involved in obesity induced cell transformation are not well understood. Leptin is an adipocyte-derived hormone/cytokine that is secreted by adipose tissue and obesity is associated with high circulating leptin levels. Beside the effects of leptin in the regulation of feeding and energy expenditure, leptin has major roles in regulating immune and metabolic responses. Recently, we have demonstrated that leptin regulates macrophage lipid metabolism through a PI3K/mTOR pathway dependent manner. Of note, mTOR is a serine/threonine kinase that plays a key role in cellular growth and is frequently altered in tumors. In this work, we investigated the role of leptin in regulating lipid metabolism, inflammation and cell proliferation in ephitelial cells. **Methods and Results:** Leptin-induced a time and dose-dependent enhanced phosphorilation of P70S6K and S6 ribossomal protein, downstream of mTOR activation. Both P70S6K and S6 phosphorilation were inhibited by rapamycin (20 nM), suggesting a role for mTOR in leptin-induced signaling in epithelial cells. IEC-6, non-transformed rat epithelial cells (5x106 per well), were stimulated with leptin (20 nM) and treated with rapamycin (20 nM), everolimus (10 nM), FK-506 (10 nM) or vehicle for 6 h. Leptin induced enhanced formation of lipid bodies (LB), dynamic organelles involved in lipid metabolism and increased human colon cancer, when compared to control cells after osmium tetroxide staining. The induction of lipid body formation was accompanied by enhanced ADRP accumulation and COX-2 expression as seen by Western blotting. We also observed that leptin was able to induce CINC-1, TGF-β and PGE2 production in these cells. Pretreatment with rapamycin significantly inhibited leptin-induced LB induction (from 9.0± 0.2 LB/cell in leptin to 4.7±0.1 in rapamycin treated cell; n=3), COX-2 expression and TGF-β production. Moreover, leptin induced the IEC cell proliferation in a mTOR-dependent manner. **Conclusion:** We demonstrated that leptin is important on the regulation of lipid metabolism, inflammatory mediator production and proliferation in intestinal cells through a mechanism dependent of activation of the mTOR pathway, thus suggesting that leptin-induced mTOR activation may contribute for the obesity-related enhanced susceptibility to colon carcinoma. Supported by: FAPERJ, CNPq, CAPES, INCT-Cancer.

10.015

Investigation of cytotoxicity of 4-nerolidilcaterol and its synthetic derivatives. Cunha CRM[1], Menegatti R[2], Valadares MC[2] [1]UFG − Farmacologia e Toxicologia Celular, [2]UFG − Química Medicinal

Introduction: 4-Nerolidilcaterol (4-NRC) is the main secondary metabolic of the Photomorphe genus. Several biological activities have been attributed to this compound, such as, analgesic, anti-inflammatory, antioxidant and antitumor. Since that the 4-NRC is a very unstable compound, we investigated the cytotoxic activity of 6 different stable synthetic derivatives. **Materials and Methods**: Using the Trypan blue exclusion method the antiproliferative activity *in vitro* of ethanolic solution 4-NRC and its 6 synthetic derivatives (LQFM001, LQFM002, LQFM006, LQFM014, LQFM015, LQFM016) were investigated on Ehrlich ascitic tumor cells, K-562 and Jurkat, after 24 hour-incubation. Afterwards, the multi-drug resistant cells K-562 were also analyzed to by MTT method, after 24 hour-incubation. **Results and Discussion:** results obtained have shown cytotoxic activities in a dependent-concentration manner showing respectively, IC50 values to each tumor lineage and derivative. To K562 lineage: LQFM 001 (IC50: 232 µM), LQFM 002 (IC50: 32.7 µM), LQFM006 (IC50: 292 µM), LQFM014 (IC50:279 µM), LQFM015 (IC50 45.2 µM), LQFM016 (IC50: 119.0 µM). To Jurkat lineage LQFM 001 (IC50: 260 mM), LQFM002 (IC50: 20.2 µM), LQFM006 (IC50: 305 µM), LQFM014 (IC50: 452 µM), LQFM015 (IC50: 38.5 µM), LQFM016 (IC50: 41.2 µM). To Ehrlich ascitic tumor cells LQFM 01 (IC50: 106.0 µM), LQFM02 (IC50: 21.7µM), LQFM006 (IC50: 367 µM), LQFM014 (IC50:108.8 µM), LQFM015 (IC50: 16.4 µM), LQFM016 (IC50: 62.12 µM). IC50 of these analogue was also found using MTT techniques to K562 lineage where the following values were observed: LQFM 01 (IC50: 287.2 µM), LQFM02 (IC50: 159.4 µM), LQFM006 (IC50:782 µM), LQFM014 (IC50:728 µM), LQFM015 (IC50 224.2 µM), LQFM016 (IC50: 74,49µM). In conclusion, we observed that the analogues LQFM 002, LQFM 015 and LQFM 016 showed higher cytotoxic activity in all tumor cell lineage investigated. These cytotoxicities were equal to those found in 4-NRC. But the analogues are more chemically stable that your parent compound. **Financial support:** CNPq, FUNEPE, FINEP; CAPES.

10.016

Effects of tumor-derived extracellular matrix on endothelial cell functions: implications to tumor-associated angiogenesis. Brandão-Costa RM[1], Saldanha-Gama RF[2], Helal Neto E[2], Morandi V[3], Barja Fidalgo TC[1] [1]UERJ − Farmacologia, [2]UERJ − Farmacologia e Psicobiologia, [3]UERJ − Biologia Celular e Genética

Introduction: Tumor progression creates a microenvironment that influences neighboring cell plasticity, migration/invasion and triggers the formation of new blood vessels. Therefore, tumors are reported to induce the formation of a new capillary network from preexisting blood vessels, a process known as angiogenesis. This process depends on the production, by tumor cells, of growth factors that stimulate new blood vessel growth and survival. Adhesion to the extracellular matrix is also important to regulate endothelial cell survival, proliferation and motility during angiogenesis. Integrins are the major protein adhesion molecules responsible for cell-matrix interactions, transducing molecular signals from microenvironment to regulate cell shape, survival and proliferation. However the contribution of tumor matrix/endothelial cell interaction to tumor-associated angiogenesis is poorly studied. In the present study, we evaluated the interactions between tumor-derived extracellular matrix and endothelial cells and how this interactions may impact tumor-associated angiogenesis. **Methods:** Tumor-associated matrix was obtained from the human melanoma cell line, MV3. Matrix derived from the melanocyte cell line NGM were used as control. For matrix characterization, NGM and MV3 were cultivated for 4 and 24 hours and the content of tenascin c, lamin, fibronectin and thrombospondin were evaluated by ELISA. The contribution of tumor matrix for endothelial cell proliferation was evaluated by MTT viability assay using the human endothelial cell line, HMEC. To investigate the contribution of specific integrins to endothelial cell proliferation, we used a panel of different disintegrins and ADAM 9. Matrix effect on endothelial cell migration was investigated in transwell experiments. Finally, we evaluated the effect of tumor-derived matrix on tubulogenesis in matrigel. **Results:** Our results indicate that, after 24 hours of culture, MV3 melanoma-derived matrix has significantly more laminin than NGM melanocyte-derived matrix. MV3 matrix substantially increases endothelial cell proliferation, which can be inhibited by incubation with ADAM 9, an inhibitor of laminin receptor. Likewise, ADAM 9 was able to inhibit endothelial cell migration induced by melanoma-derived matrix. Finally, we observed the formation of a significantly higher number of tubular structures by endothelial cells cultured on melanoma-derived matrix. Conclusions: These data suggest that the tumor-derived extracellular matrix influences endothelial cell functions and may play an important role in tumor-associated angiogenesis. **Financial support:** FAPERJ/CAPES/CNPq/UERJ

10.017

Withaphysalin F induces apoptosis and necrosis in HER-2 overexpression breast cell line. Montenegro RC[1], Rocha DD[1], Rodrigues FAR[1], Lima PDL[4], Pessoa CO[2], Maia I[3], Pessoa ODL[3], Moraes MO[1] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Oncologia Experimental, [3]UFC − Química Orgânica, [4]UEPA – CCBS

Introduction: Withasteroids are steroidal lactones (C-28) with an ergostane skeleton type, rich in oxygenated functions which are responsible for several natural modifications of the carboxylic skeleton, as well as the side chain, resulting in compounds structurally diverse and complexes. In the search of new anti-cancer drugs, withaphysalins isolated from the leaves of *Acnistus arborescens* (Solanaceae) were studied for its cytotoxic and antitumor activity. Breast cancer is one of the most incident cancers in woman worldwide, being the c-erbB2 receptor, one of the genes responsible for breast cancer metastasis and recurrence. In this way, the aim of this work is to evaluated the cytotoxicity of Withaphysalin F in erbB2-transfectec cells (high and moderate erbB2 expression) and parental cell. Methodology: The compound was tested against three cell lines: HB4a (lumial breast cell - Parental), HB4a C5.2 (HER-2 overexpression), HB4a C3.6 (HER-2 mild expression) using MTT assay, after 24, 48 and 72 hours of incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. To further understand the mechanism underlying the cytotoxicity of Withaphysalin F, studies involving membrane integrity, DNA fragmentation, cell cycle analysis, were performed by flow cytometry in HB4 C.5 cell based in IC50 values after 24h incubation. Results and **Discussion:** The tested compound showed IC50 values in the range from 12 down to 3 mM in parental cell and 6.5 down to 1mM in HB4 C 5.2, after 24h and 72h of treatment, respectively. The IC50 value after 24h incubation was 2 times lower in HB4a C 5.2 cells when compared to parental cell. HB4a C 5.2 cells treated with 2, 4 and 8 mM reduced cell viability as demonstrated by membrane integrity test, caused DNA fragmentation and cell cycle changes in a dose-dependent manner, with a discrete arrest on G2/M on cells treated with 2 mM. Also, differential morphology indicated apoptosis and necrosis induction. These findings points out to the potential of these withasteroids as model molecules to new compounds with anticancer properties on cells with HER-2 overexpression. Supported by: CNPq, CAPES, CNPq/Neoplasias, FUNCAP and InCb.

10.018

5-hydroxy-2-(4-methylphenylthio)-1,4-naphtho-quinone, a juglone derivative, induces apoptosis and necrosis in hl-60 cell line. Araújo AJ[1], Montenegro RC[1], Marinho-Filho JDB[1], Rocha DR[2], Souza ACG[3], Pessoa C[1], Costa-Lotufo LV[1], Ferreira VF[3], Santos WC[4], Moraes MO[1] [1]UFC − Fisiologia e Farmacologia, [2]UFF − Instituto de Química, [3]UFF − Química Orgânica, [4]UFF − Farmácia e Administração Farmacêutica

Introduction: Naphthoquinone compounds possess various biological activities. An interesting sub-group of naphthoquinones is the 5-hydroxy-1,4-naphthoquinone. A number of 5-hydroxy-1,4-naphthoquinones such as juglone and plumbagin display potent biological properties including antimalarial activity as well as antibacterial and cytotoxic properties, although its exact mechanisms are still unclear. The aim of this work was to evaluate the cytotoxicity of 5-Hydroxy-2-(4-methylphenylthio)-1,4-naphtho-quinone (compound 1), a juglone derivative. Methodology: The compound was tested against four cancer cell lines: HL-60 (leukemia), MDA/MB-435 (melanoma), HCT-8 (colon) and SF-295 (brain) using MTT assay, after 72 hours of incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. To perform the hemolytic assay, a 2% mouse erythrocyte suspension was used. After incubation for 1h with compounds (0.78-200 µg/mL), the supernatant containing hemoglobin was measured at 540 nm. To further understand the mechanism underlying the cytotoxicity of compound 1, differential morphology tests with May-Grünwald Giemsa and acridine orange/ethidium bromide staining were analysed and studies involving membrane integrity, DNA fragmentation, cell cycle analysis, mitochondrial depolarization, were performed by flow cytometry in HL-60 cell line based in IC50 values after 24h incubation, using beta-lapachone as a positive control after 3h and 24h of incubation. Results and **Discussion:** The tested compound was cytotoxic against three of the four cell lines tested, showing IC50 values in the range from 2.2 up to 10 mM in in HL-60 and MDA-MB-435, respectively, after 72h of incubation. The IC50 value of 24h incubation was 1.3mM in HL-60 cells. No lytic effects were observed. HL-60 cells treated with compound 1 reduced cell viability as demonstrated by membrane integrity test, caused DNA fragmentation and mitochondrial depolarization after 3 and 24h of incubation, however no cell cycle changes were observed. Also, differential morphology staining indicated that compound 1 induces apoptosis and necrosis in HL-60 cells. These findings point to the potential of these synthetic quinones as model molecules to produce new compounds with anticancer properties. Supported by: CNPq, IM/INOFAR, CAPES, CNPq/Neoplasias, FUNCAP, FINEP and InCb.